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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit : 1637
Examiner : Teresa Strzelecka
Serial No. : 10/583,088
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Inventors : Marie-Philippe BIRON

Title : OLIGONUCLEOTIDES FOR THE DETECTION OF HEPATITIS B VIRUS

DECLARATION OF MARIE-PHILIPPE BIRON UNDER RULE 132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir :

I, Marie-Philippe BIRON, Ph.D., residing at 53 rue du Château, 92600 Asnieres (France), declare and say that:

I am citizen of France.

I hold a Master degree in Biochemistry in 1983 from the Pierre and Marie Curie University in Paris, France and a Ph.D. degree in Biotechnology in 1988 from the University of Technology in Compiègne, France.

Between 2000 and 2009, I have been employed by the Bio-Rad Company as a project leader where I developed the oligonucleotides for the detection of hepatitis B virus project.

I am the inventor named in the above-identified US patent application and I am thoroughly familiar with the subject-matter described and claimed therein.

In conjunction with the present application, I reviewed the Official Action dated January 18, 2011, along with the cited prior art references. I am aware that the Patent Office considers that the present invention is obvious over the documents Saito et al., Heid et al., Higashi et al., Stoll-Becker et al., Su et al., Buck et al. and the

GenBank sequence No. X98077. However, it is my opinion that these references do not disclose or suggest the present invention, as will be further explained below.

Indeed, the primers of sequence SEQ ID NO: 2 and SEQ ID NO: 3 can detect any variant of HBV (genotypes A to G) with good detection sensitivity (DNA concentrations of HBV between 100 and 10^9 copies / ml can be quantified, as described in the present application at page 14, line 25).

The cited documents describe primers hybridizing to close regions of the HBV genome. However, these documents do not validate the sensitivity of the detection method using the primers they describe over a wide range of HBV DNA concentrations. Indeed, the selective values detected by Saito et al. vary only between 250 and 2600 copies / ml (Figure 1, page 328), sensitivity of the detection methods used by Higashi et al., Stoll-Becker et al. and Su et al. have not been studied by the authors, and Heid et al., Buck et al. and GenBank No. X98077 do not describe probes and primers to detect HBV.

Moreover, even if the cited documents had shown that the primers they describe could detect HBV variants with good detection sensitivity, these results would not have allowed predicting the sensitivity of detection of the primers of the invention, as the sequences of the primers of the invention are different from that described in the cited documents.

Finally, I have produced comparative data over the document WO 93/13120 which was cited in the International Search Report of the PCT application related to the present application. These data show that primers hybridizing to overlapping regions of the HBV genome yet yield significant differences in sensitivity (see below).

a) Data concerning SEQ ID NO: 2

The following oligonucleotides have been compared:

Name	Sequence	Position
SEQ ID NO: 2	**GCTGAATCCCGCGGACGA**	1440-1457
Sequence 2a	GGC**GCTGAATCCYGCGGACGA**CCCBTCTCG 3' Y = C/T, B = C/T/G	1437-1466

Sequence 2a corresponds to the sequence SEQ ID NO: 13 described in WO 93/13120.

Accordingly, real-time PCR experiments have been conducted with:

(i) a first primer consisting of either SEQ ID NO: 2 of the present application or Sequence 2a

(ii) a second primer consisting of SEQ ID NO: 3 of the present application, and

(iii) a probe consisting of SEQ ID NO: 12 of the present application.

The targets used were the HBV genotype A Accurun® panel diluted from 10^6 to 50 copies of HBV/ml, and HBV genotypes A to G diluted at a theoretical concentration of 10^3 copies of HBV/ml. The probe was linked on the 5' terminus by FAM and on the 3' terminus by dabcyl.

	SEQ ID NO: 2		Sequence 2a	
HBV DNA copies/ml	Mean Ct	Mean Maximum Fluorescence	Mean Ct	Mean Maximum Fluorescence
1.10^6	25,2	0,603	23,4	0,900
1.10^5	29,9	0,501	27,6	0,715
1.10^4	31,7	0,401	31,3	0,643
1000	35,4	0,361	34,3	0,575
100	37,4	0,271	37,0	0,408
50	39,5	0,225	39,5	0,336
None	0,0	0,006	0,0	0,006
Coefficient of correlation	0.979		0.985	
Slope	-3.08		-3.35	

	Log (copies/ml)		
	Theorical	Measured	
Genotype		SEQ ID NO: 2	Sequence 2a
A	3	3.73	3.58
B	3	3.34	3.02
C	3	4.56	4.35
D	3	2.92	2.88
E	3	2.85	2.88
F	3	4.18	3.95
G	3	2.94	3.77

Similar results were obtained for primers on both targets. However, whereas for genotypes A to F, the concentrations measured with SEQ ID NO: 2 and Sequence 2a are not considered significantly different (the difference between the 2 concentrations is less than 0.5), quantification of the genotype G is significantly different using SEQ ID NO: 2 and Sequence 2a, the value obtained with SEQ ID NO: 2 being the closest to the expected concentration.

Sequence 2a is very close to SEQ ID NO: 2. In fact, it fully includes SEQ ID NO: 2 and only differs from SEQ ID NO: 2 by the presence of 3 extra nucleotides in 5' and 9 additional nucleotides in 3'. These 'extra nucleotides' correspond to the nucleotides flanking the sequence SEQ ID NO: 2 in the HBV genome of sequence SEQ ID NO: 1. The sequences SEQ ID NO: 2 and Sequence 2a are therefore both complementary sequences of the HBV genome and they hybridize to a single common region on the genome of HBV. However, SEQ ID NO: 2 has unexpected and advantageous properties compared to Sequence 2a.

b) Data concerning SEQ ID NO: 3

The following oligonucleotides have been compared:

Name	Sequence	Position
SEQ ID NO: 3	**GTGCAGAGGTGAAGCGAAGTG**	1582-1602
Sequence 3a	ACGTGCAGAGGTGAAGCGAAGTGCACACGGTCCGGCAGATGAGAAGGC	1580-1627

Sequence 3a corresponds to the sequence SEQ ID NO: 13 of document U.S. 5,877,162 which was cited in the International Search Report of the PCT application related to the present application.

Real-time PCR experiments as described above have been conducted with:

(i) a first primer consisting of SEQ ID NO: 2 of the present application,

(ii) a second primer consisting of SEQ ID NO: 3 of the present application or Sequence 3a, and

(iii) a probe consisting of SEQ ID NO: 12 of the present application.

The results obtained indicate that Sequence 3a is not suited for real-time PCR:

	SEQ ID NO: 3		Sequence 3a	
HBV DNA copies/ml	Mean Ct	Mean Maximum Fluorescence	Mean Ct	Mean Maximum Fluorescence
1.10^5	28,5	0,530	40,7	0,060
1.10^4	32,6	0,459	42,0	0,046
1000	36,2	0,351	41,8	0,055
100	41,5	0,194	44,5	0,045
50	42,6	0,162	44,5	0,044
None	0,0	0,003	0,0	0,007
Coefficient of correlation	0.900		0.265	
Slope	-4.97		-1.09	

Indeed, no coherent result (coefficient of correlation of 0.265) could be obtained using Sequence 3a for the HBV genotype A Accurun® panel dilution.

In addition, no result could be obtained for the detection of genotypes A to G with Sequence 3a, while SEQ ID NO: 3 yielded results close to the expected values:

	Log (copies/ml)		
	Theorical	Measured	
Genotype		SEQ ID NO: 3	Sequence 3a
A	3	3.86	-
B	3	3.65	-
C	3	4.52	-
D	3	3.46	-
E	3	3.36	-
F	3	4.08	-
G	3	3.41	-

Sequence 3a is very close to SEQ ID NO: 3. In fact, it fully includes SEQ ID NO: 3 and differs from SEQ ID NO: 3 by the presence of 2 extra nucleotides in 5' and 25 additional nucleotides in 3'. These 'extra nucleotides' correspond to the nucleotides flanking the sequence SEQ ID NO: 3 in the HBV genome of sequence SEQ ID NO: 1. The sequences SEQ ID NO: 3 and Sequence 3a therefore hybridize to a single common region on the genome of HBV.

Given the results obtained with Sequence 3a, it is completely unexpected that SEQ ID NO: 3 is suitable for real-time PCR. SEQ ID NO: 3 has therefore unexpected properties.

In conclusion, all these comparative data show that a primer including the sequence of another primer, while being complementary to the genome to be amplified, may lead to different and less advantageous amplification results. Thus, two sequences close to each other do not have intrinsically the same properties.

The primers described in the cited documents are far more remote from SEQ ID NO: 2 and SEQ ID NO: 3 than the sequences Sequence 2a and Sequence 3a

which have been assayed for comparative purposes and which include SEQ ID NO: 2 and SEQ ID NO: 3.

Therefore, I believe that nothing in the cited documents allowed predicting that SEQ ID NO: 2 and SEQ ID NO: 3 could detect any variant of HBV with good detection sensitivity. Consequently, I believe that the sequences SEQ ID NO: 2 and SEQ ID NO: 3 involve an inventive step over the sequences described in the documents of the art.

The undersigned Declarant declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed on May 24th, 2011

Marie-Philippe BIRON, inventor